MORPHOLOGY OF GLOCHIDIA OF *LAMPSILIS HIGGINSI* (BIVALVIA: UNIONIDAE) COMPARED WITH THREE RELATED SPECIES

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ABSTRACT

Glochidia of the endangered unionid mussel *Lampsilis higginsi* (Lea) are morphologically similar to those of several other species in the upper Mississippi River. Life history details, such as the timing of reproduction and identity of host fish, can be readily studied if the glochidia of *L. higginsi* can be distinguished from those of related species. We used light and scanning electron microscopy and statistical analyses of three shell measurements, shell length, shell height, and hinge length, to compare the glochidia of *L. higginsi* with those of *L. radiata siliquoidea* (Barnes), *L. ventricosa* (Barnes), and *Ligumia recta* (Lamarck). Glochidia of *L. higginsi* were differentiated by scanning electron microscopy on the basis of a combined examination of the position of the hinge ligament and the width of dorsal ridges, but were indistinguishable by light microscope examination or by statistical analyses of measurements. Analysis of variance and multivariate (principal component) analysis separated *L. radiata siliquoidea* from the other three species by virtue of its larger size, but discriminant function analysis classified only 38% of the glochidia of *L. higginsi* correctly compared with 83% of those of *L. radiata siliquoidea*.

The glochidia of most unionid freshwater mussels are obligate parasites on the gills or fins of fishes. Glochidia discharged from the marsupial gills of females attach and encapsulate on fish and undergo organogenesis to the juvenile stage (Coker *et al.*, 1921). Information on the life history and recruitment of mussel species can be readily developed by the collection and identification of glochidia. For example, Zales and Neves (1982a, b) using light microscopy, determined the timing of glochidial release, periods of infection, and the identity of fish hosts for four lampsiline mussels by collecting and identifying glochidia in stream drift and on fish gills.

Glochidia of the endangered *Lampsilis higginsi* (Lea) are morphologically similar to those of several other species of Lampsilinae in the upper Mississippi River (Surber, 1912, 1915). Before information about the reproductive cycle and host fishes could be determined, a method for operational/field identification of the glochidia of *L. higginsi* was required.

Several methods have been used to study glochidia. Shell shape and gross features have been described by light microscopy (Lefevre and Curtis, 1910; Surber, 1912, 1915; Utterback, 1933; Inaba, 1941), shell dimensions have been measured (Surber, 1912, 1915; Inaba, 1941; Wiles, 1975; Zale and Neves, 1982a), and scanning electron microscopy has been used by several researchers (Heffelfinger, 1969; Calloway and Turner, 1978; Clarke, 1981, 1982; Rand and Wiles, 1982). Although Surber (1912) provided camera lucida drawings and measurements of glochidial length and width from samples of *Lampsilis higginsi*, he provided no definitive identification of the species. No further descriptions of *L. higginsi* glochidia have been reported.

Our objective was to ascertain simple techniques that could be used routinely in the field, including light microscope examination and measurements of shell dimensions, to differentiate the glochidia of *Lampsilis higginsi* from those of three other lampsiline mussels *(L. radiata siliquoidea* (Barnes), *L. ventricosa* (Barnes), and *Ligumia recta* (Lamarck)

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in the upper Mississippi River system. In addition, scanning electron microscopy was used to study aspects of the comparative ultrastructure of the shells of these four species.

MATERIALS AND METHODS

Gravid females of 15 species of mussels, in addition to *Lampsilis higginsi*, were collected from the upper Mississippi River (Pools 7 and 10) by handpicking and brailing. After preliminary examination, we selected *L. radiata siliquoidea* (here termed *L. radiata*), *L. ventricosa*, and *Ligumia recta* for detailed study because of the close similarity of their glochidia to those of *L. higginsi*. We removed glochidia from live females by using a hypodermic needle and syringe to flush the marsupial portion of the gill. Glochidia that were infective and therefore selected for examination responded by snapping their valves shut when placed in a 1.0% NaCl solution. Other glochidia came from females preserved in 10% formalin or 70% ethanol. In measuring length (maximum anterior-posterior), height (maximum dorsal-ventral), and hinge length, we examined 20 glochidia per female under a microscope (100x) fitted with an ocular micrometer.

Data analyses were conducted using the Statistical Analysis System (SAS Institute, 1979) at Iowa State University, Ames. Statistical significance is defined as $P < 0.05$.

Photographs were taken of representative specimens of glochidia of each species for qualitative comparisons of general shell features. All aspects of the shell were photographed, including lateral views showing the shape of the shell and hinge, and the position, size, and shape of adductor muscle, a dorsal view showing the hinge and beak sculpture, and an anterior-posterior view showing the flange and shell gape.

Some glochidia of each species were fixed in 10% buffered formalin and held in 70% ethanol for scanning electron microscopy. Samples were prepared by critical point drying and sputter coating with platinum palladium (Postek *et al.*, 1980). Shells were studied at magnifications of 300x to 10,000x.

RESULTS AND DISCUSSION

STATISTICAL ANALYSIS

One-way analyses of variance revealed that overall significant differences existed among glochidia of the four species in the three morphometric characteristics measured. However, the source of the difference was not due to *Lampsilis higginsi*, but to *L. radiata* which was significantly greater in length, height, and hinge length than the other three species (which did not differ significantly from one another). (Table 1.) In addition, a multivariate (principal component) analysis also did not separate *L. higginsi* from the other species (Fig. 1). The first principal component had similar loadings for all three characteristics (height = 0.59, length = 0.60, hinge length = 0.54) and accounted for 77% of the total variance in the correlation matrix. Component 2 (hinge length = 0.83, height = 0.47, length = 0.29) accounted for only 16% of the variance. Again, *L. radiata* could be separated from the other three species by its larger size, but *L. higginsi* did not differ significantly from *L. ventricosa* and *Ligumia recta*.

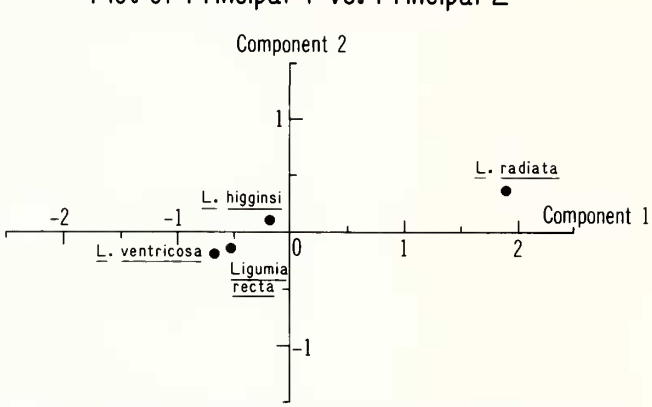

Fig. 1. Principal component analysis. The large size of *Lampsilis radiata* glochidia separates it from the other forms along component 1.

Glochidia of *Lampsilis higginsi* were correctly classified in 39% of the observations by discriminant analysis, but 55% were misclassified as either *L. ventricosa* or *Ligumia recta* (Table 2). Correct classifications were 50% for *L. ventricosa* and 48% for *Ligumia recta*. Discriminant function analysis was the most accurate for glochidia of *L. radiata* correctly classifying 83% of the glochidia, 10% were misclassified as *L. higginsi*.

LIGHT MICROSCOPY

The shape and appearance of shells of the four species examined were so similar that identification by observation with the light microscope was not possible (Fig. 2). Our general observations of the shells were similar to those of Lefevre and Curtis (1910) for hookless glochidia in shape of the shell, the double margin around the periphery of the shell, granulations on the lateral surface, the position and shape of adductor muscle, and the presence of two pairs of microprojections. When profiles of the shells of each species were compared by overlaying transparencies of shells of the same size, no obvious differences in shape could be detected, although about 4% of the glochidia in one female *Ligumia recta* were much higher than most glochidia of this species (height $\bar{x}$ = 296 μm). The relative position of the hinge ligament could be discerned in some glochidia of each species at 40x. The hinge ligament in *L. recta* was centrally located whereas that in the three *Lampsilis* species was more posterior. The position of the adductor muscle was not considered for use in identification because the larval adductor muscle is lost soon after a glochidium attaches to a fish. Other features of the glochidium were not adequately resolved by light microscopy to be useful for species identification.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy showed all four species have similar surface features. A series of semi-circular ridges

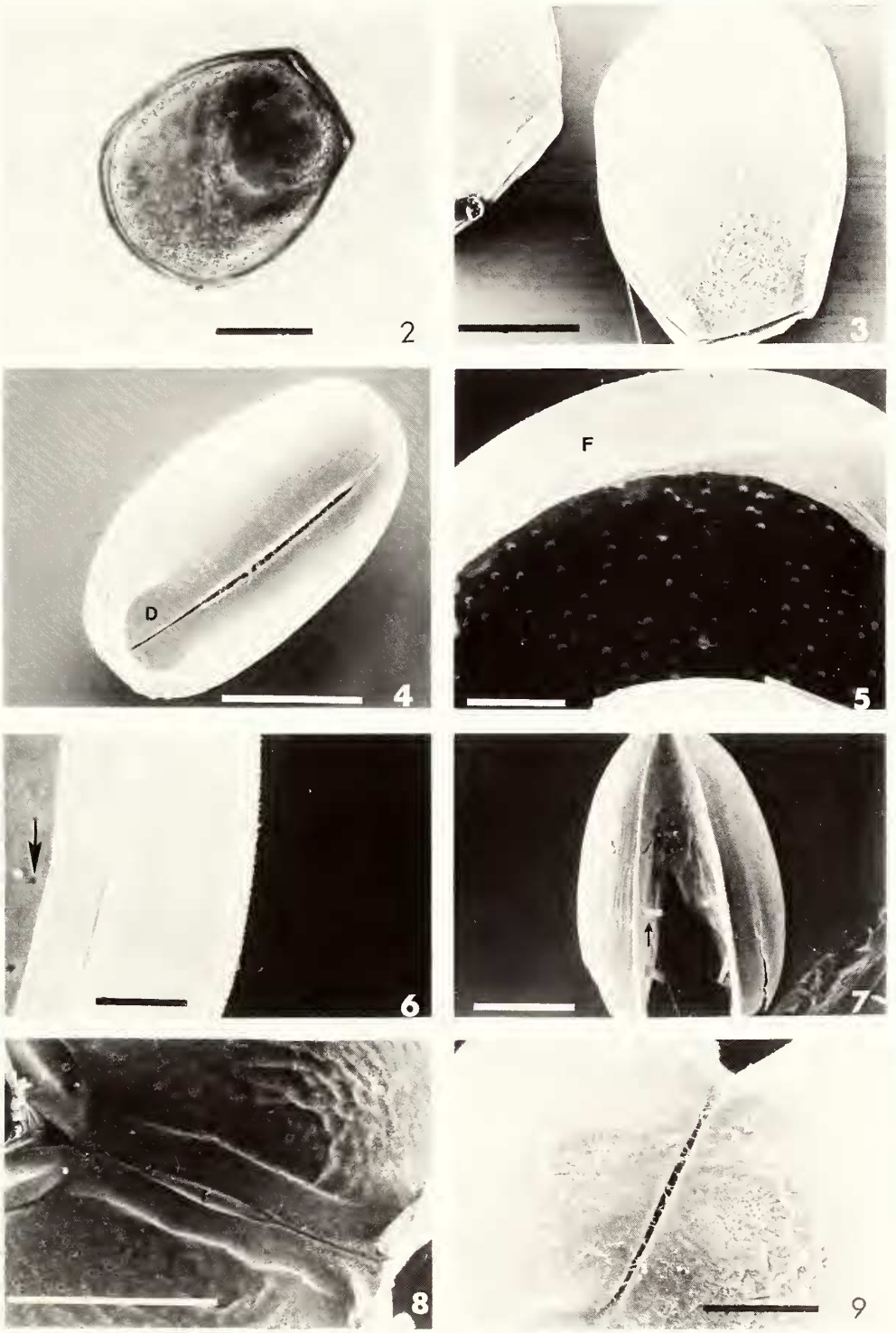

Fig. 2. Glochidia of *Lampsilis higginsi:* light microscope photograph (scale bar = 100 μm). **Fig. 3.** Scanning electron micrograph of characteristic features of shell valve of Lampsilinae glochidia (scale bar = 100 μm). **Fig. 4.** Scanning electron micrograph (anterior view) showing flattened dorsal ridges (D) of *Lampsilis higginsi* (scale bar = 100 μm). **Fig. 5.** Ventral flange (F) and a portion of the lateral shelf of glochidium of *L. ventricosa* (scale bar = 20 μm). **Fig. 6.** Ventral flange showing fine tooth-like projections and pits on internal surface (arrow) of glochidium of *L. ventricosa* (scale bar = 10 μm). **Fig. 7.** Internal view of the glochidium as seen in the gaping shell: mantle, adductor muscle, and microprojections (arrow) (scale bar = 100 μm). **Fig. 8.** Internal view of hinge ligament, placed slightly posterior in *Lampsilis radiata* (scale bar = 100 μm). **Fig. 9.** External sculpturing of the shell at the dorsal edge in *Lampsilis ventricosa* glochidium (scale bar = 50 μm).

Table 1. Mean measurements (standard deviations in parentheses) of glochidia of four Lampsilinae mussels. Means for each measured characteristic with the same superscript are not significantly different from each other (P = 0.05) (Student-Newman-Keul's test of means).

Species	Number of glochidia	Number of females	Measurements (μm)		
			Height	Length	Hinge
Lampsilis higginsi	96	3	259[a] (8.0)	215[a] (4.2)	110[a] (4.2)
L. radiata	220	11	271[b] (1.2)	228[b] (0.8)	120[b] (0.5)
L. ventricosa	556	19	257[a] (0.9)	216[a] (0.8)	107[a] (0.5)
Ligumia recta	180	9	259[a] (0.9)	213[a] (0.5)	107[a] (0.5)

on the lateral surface become wrinkled near the hinge (Fig. 3). In addition, each valve has many pits on both the internal and external surface, which have sometimes been interpreted as pores (Arey, 1924; Zs.-Nagy and Labos, 1969; Calloway and Turner, 1978; Rand and Wiles, 1982). When viewed from the lateral external surface, the shell does not appear to be porous, but in cross-sectional and internal examinations of the valves, the pits appeared to be continuous. This apparent discrepancy could have been explained by Calloway and Turner (1978), who noted that the external surface appeared perforated only at accelerating voltages of 20 kilovolts and greater. They concluded that the periostracum was not perforated and that the appearance of pores on the outer surface was an artifact of the scanning electron microscope. Perhaps electrons penetrate the periostracum at 20 kilovolts making it appear transparent and the pits appear as pores. Posterior and anterior edges of each valve are flattened near the dorsal aspect, forming a smooth surface about 65 μm long (dorso-ventral) and 25-40 μm wide (medial-lateral) here referred to as dorsal ridges (Fig. 4). The peripheral edges of the valves are turned inward to form a continuous shelf around the inner margin. Ventrally, the shelf forms a flange or lip believed to be analogous to the hook of anodontine mussels described by Lefevre and Curtis (1910) (Fig. 5). The flange is about 13-19 μm wide and extends the width of the ventral shell margin. Fine, tooth-like projections, previously described as microstyles (Clarke, 1985), cover all except the proximal one-third of the flange (Fig. 6). The microstyles decrease in length to micropoints on the inner edge of the flange. In all four species, the microstyles are arranged in irregular vertical rows and about 14-17 rows cover the flange from the inner to the outer edge. The inner shell margin provides an attachment site for the mantle, a thin sheet of tissue covering the inner valve surface except in the region of the adductor muscle. The single adductor muscle was also seen internally near the dorsal margin. A pair of cylindrical microprojections, about 24-26 μm long, previously described as "sensory hairs" (Lefevre and Curtis, 1910), is near the ventral margin of the valve (Fig. 7). At the dorsal edge of the valve, the shelf folds inward forming an articulating surface for junction of the valves. The larval ligament connects the valves at this hinge line (Fig. 8).

Table 2. Summary of percent of each species classified as either *Lampsilis higginsi, L. radiata, L. ventricosa*, or *Ligumia recta* by discriminant analysis.

Known species	Percentage classified into species				
	Lampsilis higginsi	*L. radiata*	*L. ventricosa*	*Ligumia recta*	Number of specimens
Lampsilis higginsi	39	6	22	33	96
L. radiata siliquoidea	10	83	5	2	220
L. ventricosa	19	8	50	24	556
Ligumia recta	20	5	27	48	180

Table 3. Width of the dorsal ridge of the four Lampsilinae species. Mean dorsal ridge widths with the same superscript are not significantly different from each other (P = 0.05) (Student-Newman-Keuls' test of means).

Species	N	Width of ridge (μm)		
		Mean	SD	Range
Lampsilis higginsi	9	27.20[a]	1.75	25.0-29.2
L. radiata	9	33.48[b]	3.13	28.0-37.9
L. ventricosa	10	34.70[b]	3.06	30.0-40.0
Ligumia recta	8	28.66[a]	0.96	25.8-30.0

We concentrated on three features of the shell in our efforts to distinguish among the species: (1) position of the hinge ligament; (2) width of the flattened dorsal ridges; (3) sculpturing on the lateral shell surface. The first two features proved to be the most useful for separating *Lampsilis higginsi.* Hinge ligaments were central in glochidia of *Ligumia recta*, whereas they were slightly more posterior in *L. higginsi, L. ventricosa,* and *L. radiata.* The dorsal ridges of each valve, measured at their greatest width, differed significantly among species (Table 3). The ridge width was usually narrower in *L. higginsi* and *Ligumia recta* (250-300 μm) than in *L. ventricosa* and *L. radiata* (280-400 μm).

The shell sculpture showed no major differences among the species, though there was some subtle variation. We attempted to identify photographs of each species on the basis of shell sculpture alone, but could not consistently detect a representative pattern on each shell.

CONCLUSION

The objective of this study was to find an operational/field method for routine identification of *Lampsilis higginsi.* Light microscopy and statistical analyses of shell dimensions were found to be inadequate for species differentiation. Scanning electron microscopy can be used to differentiate glochidia

of *L. higginsi* from the other three species on the basis of the position of the hinge ligament and the width of the dorsal ridges, but the technique is expensive and impractical for identification of small samples of glochidia collected in the field. The technique could be of use when there is justification for a significant investment of time and expense in identification of glochidia. Hoggarth and Cummings (pers. comm.) used scanning electron microscopy to identify glochidia of *Anodonta grandis grandis* Say on fish in field collections and suggested this technique was more labor efficient than artificial infection experiments for determining host fishes. On the contrary, we have found that artificial infection requires much less equipment, training, and expense than scanning electron microscopy and is more practical for routine use.

Laboratory culture of glochidia and juveniles (Isom and Hudson, 1982; Hudson and Isom, 1984) could be another route for developing early life histories. Investigators could follow the growth of a mussel and document developmental stages at which *Lampsilis higginsi* can be positively differentiated from related species by light microscopy. One could then verify fish hosts by holding field-collected fish in the laboratory until juvenile mussels have dropped off and developed into an identifiable stage. Recruitment of a species could also be evaluated by identification of juveniles in the field.

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